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# KANSAS UNIVERSITY SCIENCE BULLETIN.

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## CYTOLOGY AND TAXONOMY.

BY C. E. McCLUNG.*
(Contribution from the Zoölogical Laboratory, No. 174.)

THERE are certain large problems in the domain of biology that confronted the earliest investigators, and these have been handed down from one generation of scientists to another. As new and more restricted departments separated themselves from the older and more general ones they carried with them these same problems, toward the solution of which they applied their own special methods, giving them at the same time something of their own bias. Very prominent among these great questions, and, in a measure, at least, embracing them all, are those which concern the relation of organisms to each other, and the processes of development in the individual organism.

To the youngest of the biological sciences have descended these heritages of the scientific ages, and now we hear it from many sources that all biological problems are to-day problems of the cell. Clearly enough it has been recognized that cytology might have much to say regarding the mechanism of ontogenetic growth, but less definitely, and only more recently, has a conception of what it might do for phylogeny arisen in the minds of biologists. It was with the thought of these two functions of cytology in mind that I chose the somewhat indefinite title for this paper. I cannot but believe that cytology will honorably fulfil the obligations that science has placed upon it, and I feel that it is indeed a privilege to stand before you and call to your attention what our young department has already been able to accomplish, and to suggest possibilities for further usefulness.

* An address delivered before the section on Cytology and Heredity of the Seventh International Zoological Congress, at Boston, August 20, 1907.

It is with no desire to magnify my own humble part in the attack upon these problems that I make mention of the manner in which they have presented themselves to me. I have thought that by means of a concrete example I might perhaps more clearly exhibit the grounds for my theoretical beliefs and the reasons for the faith that is within me. I shall therefore first indicate the main features of parallelism between germ-cell structure and taxonomic relations that have appeared from investigations upon certain orthopteran species and then attempt explanations of the causal connections between the two sets of phenomena in the light of our present general knowledge.

From the study of a large number of saltatorial orthopteran species it appeared that the normal numbers of chromosomes in the males fell, in general, into two groups of twenty-three and thirty-three respectively. To a cytologist this was a very suggestive fact, for it indicated a precision in the organization of cells that was in advance of anything that had before been imagined. It was doubly interesting to note the opinions of orthopteran taxonomists with regard to the relationships of these species and to discover that they had segregated them into definite corresponding groups which are called "families." Merely as the result of the study I had made of the germ-cells, I would have classified these insects into two groups, one having a complex of twenty-three chromosomes and the other of thirty-three. On the other hand, many taxonomists, from careful and minute examination of the external anatomy of these same species, had agreed in placing them into family groups, which they call the "*Acrididæ*" and "*Locustidæ*." Now, these families are clearly distinguished apart by ordinary taxonomic features, and to speak of the "short-horned" and "long-horned" grasshoppers is to summon before the mind of one acquainted with the animals very definite types of structure. And yet the distinction between these insect families is no more apparent to me from an inspection of the gross anatomy than it is from a study of the germ-cells. The possession of a complex of twenty-three chromosomes is just as typical an acridian character as is that of short antennæ. I do not regard it any argument against this cytological means of discrimination to learn that other than acridian organisms have twenty-three chromosomes, for so do other animals have short antennæ.

Clearly enough, it would seem, we have here an indication of

the great precision in organization so long inferred for the germ-cells. Were our knowledge more extensive we should doubtless see yet further evidences of this—and certainly it is our duty to seek them out—but for the present we must make use of this suggestive fact and learn from it what we may. The most important conclusion that can be drawn is that these chromosomes are primary factors in the mechanism of development, for no other structures of the cell exhibit any such constancy and definiteness of organization to parallel the structural differences of the resulting animals. Since they occur in the same number throughout the family, and since the end result of their changes is essentially the same in every case, they must be individually alike in each complex. Further, it may be concluded that always their functions for any given period in ontogenesis are the same. With this as a beginning it becomes a reasonable hope that we may attempt an analysis of the processes of organic development by associating modifications of the acridian type of body with variations in the chromosome complex of their germ-cells. In other words, the task of the cytologist engaged in the study of this group is to learn the history of the individual chromosomes and to associate this knowledge with what is known regarding the development of somatic structures.

This is obviously no light task, but, on the other hand, I do not consider it an impossible one. Fortunately, the chromosomes of different species have individual peculiarities in size, shape and behavior that make it possible to identify many of them without difficulty. Whether it will be possible to homologise them throughout the family is not, of course, nearly so certain. Such an extensive and thoroughgoing attack upon the problem may, however, not be entirely necessary. It may be sufficient to attempt an analysis of a more limited group than the family. In that hope a great many genera have been studied in order to find striking peculiarities that might assist in the establishment of relationships between chromosomes and somatic characters. The work has thus, for a time, been narrowed down to a few forms; and since my desire in this paper is not to give detailed results, but rather to point out the direction in which, I believe, work of this character should proceed, and to indicate the essential nature of my conclusions, I will briefly describe the conditions of the investigation at the present time.

Students of the Orthoptera have divided the family *Acrididæ* into nine subfamilies, of which only four are found in the United States. Material from these four has been studied, and I feel convinced that one of these subfamilies should be removed from the group. The other three are manifestly related, and so closely do two of them grade together that there is much dispute as to the disposition of certain genera. The third subfamily is definitely marked, and it is sufficient for the systematist to find a distinct spine on the prosternum of an acridian in order to place it in the subfamily *Acridiinæ*. Within this division of the family there are a large number of subgroups, the principal one being the Melanopli. This includes many extensive genera, of which one, *Melanoplus*, contains over 120 species in the United States. Another genus of this group, *Hesperotettix*, is of wide distribution but embraces only seven species. Of these latter I have studied three and they seem to be plainly marked in their body characters. I have thought that, by selecting a variable genus and another of the same group that is more stable, it might be possible to determine the general nature of the changes associated with variability. Both of these lines of investigation are being pursued, but it seemed best to consider first the nature of the less variable form, particularly since there is one chromosome that can definitely be identified throughout the genus.

This latter circumstance I consider extremely fortunate, for the occurrence of the same number of chromosomes within the family raises the presumption that they are serially homologous almost to a certainty; the discovery of the same element in three species of a certain genus amounts to a positive proof of this fact. Particularly is this true when it is understood that the homologous elements really represent two of the chromosome complex. With this definite structure indubitably marked in the different species, I felt that I had opened up before me an opportunity of unusual promise, but one which would require long and tedious labor for its development. I regret that more has not been accomplished, but what has been done is sufficient to indicate the fruitfulness of the investigation.

Briefly, then, we may note that, so far as our observations have gone, all the species of the genus *Hesperotettix* have within their first spermatocytes a multiple chromosome, a hexad, consisting of the accessory chromosome and one of the

tetrads. This is a generic character, one of those that serve to distinguish the group that taxonomists have called *Hesperotettix* from other members of the family *Acrididæ*. With my present knowledge I would feel safe, if I found an acridian cell containing such an element, in ascribing its source to the genus *Hesperotettix*. I should have as little doubt regarding its distinctive character as I should have regarding that of the animal from which it came. So far as I have been able to see, there is no other character of the cell that would distinguish this genus from the remaining ones of the *Acrididæ*.

Then it became necessary to see if there were peculiarities that bore any constant relation to body characters of specific value. It was most interesting to find that the multiple chromosome, so constant a generic character, should exhibit just as constantly minor modifications of size and proportion in the different species. This particular chromatic element therefore signifies to my mind as definite an integration of substance as does the adult animal which contained it. The differences between the same element in the three forms of *Hesperotettix* studied are just as specific as any that might be chosen from somatic characters. It would perhaps be well to say that I have made most careful comparisons in the size of these chromosomes, and it is very remarkable to find the extremely close concordance that exists within the species. If this is true of the fixed material which has been subjected to all the violent processes incident to the preparation of slides, how much more true it must be for the living object.

There are thus within the germ-cells of these animals certain structures that show specific, generic, and family characters in just as pronounced a manner as do the completed organisms wherein they are found. It is therefore desirable to know what corroboration we have from the study of other organisms for our belief in the great importance of the chromosomes. I would ask you, therefore, to review with me very briefly a series of facts which cytology, normal and experimental, has brought forth in support of the idea that the chromatin is the guiding and controlling element in development, and that the chromosomes represent definite centers of influence.

In the first place, organisms without chromatin do not exist, and when the nucleus is removed from an individual the normal functions cease. The necessity of the chromatin in the economy of the cell is thus proved. Next we may note that the

integration of the chromatin is always into a definite number of chromosomes in the individual, in the species, and sometimes in an entire family. From this the precision of organization inherent in the chromatin may be inferred. Again, throughout all the complications of the mitotic division every effort is apparently put forth by the cell to secure an accurate division of the chromatin. This suggests the primary importance of the chromatin and hints strongly of differential organization.

If now we turn from cells in general to the special category of germ-cells we find very much more evidence for our belief that the chromosomes are the determinants of characters and that they are qualitatively different. From this source we learn that normally, for the production of a new organism, two cells are required, and that the only feature of equivalence between them is in the chromosome complex, which in each case is a duplicate of the other. Not only are these morphologically equivalent groups of chromosomes, but they are also physiologically equal, for either complex alone may condition development in the same way, as is shown by parthenogenesis and merogony. Further evidence of this independence and equivalence of the chromosomes is furnished by the history of the cells of which they are a part, for step by step they go through the same preparation of maturation, and the particulars wherein they show resemblances are always those of chromosome behavior, no matter how much the other elements of the cell may differ.

While either the paternal or maternal chromosome complex in a cell is sufficient to condition the development of all the characters of the organism, the absence of any one chromosome of the group results in the non-development of some body characters. Such, at least, seems to be the conclusion that must be drawn from the work upon polyspermized eggs. It therefore appears very probable that the chromosomes are qualitatively different. Very strong additional evidence to support the view that the chromosomes *are* qualitatively different, and a duplicate series when present in the normal number, is afforded by the parallelism between the behavior of the chromosomes in maturation and fertilization, and the segregation of Mendelian characters in hybrids.

Let it be granted from the facts adduced that the chromosomes are the controlling factors in development, then how can we explain their action, how correlate them individually with

the somatic characters, and how explain their differences in the formation of body-cells and germ-cells from the same fertilized egg? Here we have the problem of heredity stated in cytological terms, and any theory offered in explanation must conform itself to the known facts. I am of the opinion that in our work upon the classification of organisms we have been much inclined to regard only the end stage of the process of development. A starfish has not been for us a starfish until it has acquired its radial symmetry and its adult organs. I believe that in this we are mistaken. The egg of the starfish performs its functions in just as specific a manner, and differs from the egg of a sea-urchin as truly, as do these same organisms differ after a few weeks of development. We must realize that an organism functions from its very beginning and that it does this differently from any other individual. We may not now be able to perceive these differences of individuals in the one-celled condition so clearly as we do when they are many-celled, but this is due to our lack of knowledge and not to the absence of variation. That one cell is sufficient for the identification of the species is clear enough from my study of *Hesperotettix* and other Orthoptera.

We must indeed recognize that organisms are specifically different throughout their ontogeny, and that they exhibit the functions of protoplasm specifically in every case—not one function, but all of them. It would accordingly be as difficult to add sex to an animal at some late stage of development as it would be for the egg starfish to change into an adult sea-urchin. The organization is inherent in the individual from the beginning; its method of expression differs, however, from stage to stage. Therefore we must conclude that the parts of the whole also differ progressively. Perception, movement, metabolism and reproduction are functions of the one cell just as truly as they are of the multitude of cells that results from its division.

If chromosomes are centers of influence governing always the manifestation of specific energies, then they must at every stage of development, both ontogenetic and phylogenetic, exhibit the same properties. In so doing they show themselves subject to the same laws of change that obtain in the differentiation of cells, for the nature and extent of their influence vary progressively. I think it is reasonable to conclude thus, for, if our assumption regarding the sex-determining nature of the accessory chromosome be correct, this is just what we

find to be true regarding the development of the sexual characteristics which are inherent in every cell of the body, and become progressively different in each state of development.

It is hardly necessary to say that no true student of cellular phenomena regards the chromosomes as the *only* factor in development. They play their part in the economy of the cell and accomplish their work solely because they exist under certain conditions. They appear to us as more definite and constant features of cell architecture than any others, and in their behavior exhibit such indications of initiative and importance as to lead to the belief that theirs is a directive action. Differentiation is regarded as a progressively changing series of interactions between the chromosomes and other parts of the cell, of such a nature that the nucleus initiates changes which are limited by the conditions of the cytoplasm.

In order that we may gain some idea of the possible nature of these interactions, I would ask you to consider with me certain phenomena that are observable in the development of the male germ-cells. Studies on spermatogenesis usually begin with a consideration of the spermatogonia, since these are the first cells found in a definite sexual organ, but there is little doubt that these are the last of a line of pure germ-cells that have been set off early in the embryonic history. These spermatogonia have the paternal and maternal chromosomes present in the same relations as exist at the time of fertilization. They divide rapidly and continuously until they become much decreased in size and almost the entire cell is nucleus. The cytoplasm is reduced to a minimum, but the nucleus, at least so far as the chromosomes are concerned, has not been much altered. In the grasshoppers, I feel convinced that the number of these divisions is, for each species, a constant. The reduction of the cytoplasm does not go beyond a certain point, and the process is self-limiting.

At this point conditions change. The members of the chromosome group, instead of remaining separate, as heretofore, unite in pairs, the components of which are size equivalents, and in all probability functional equivalents, from the two parents. This step is one toward which the chromosomes seem to have been tending during the later spermatogonial divisions, and one which certain elements anticipate in some species. There is every reason to suppose that this synapsis is a union of homologous elements, and is the consum-

mation of the fertilization process, initiated by the union of the spermatozoon and the ovum and rendered more intimate by the fusion of the nuclei. There is not entire agreement among observers regarding the exact time of the synapsis of the chromosomes, but it is always described as occurring between the last spermatogonial division and the first spermatocyte mitosis. In the grasshopper it is the final act of the spermatogonial chromosomes, and precedes the changes of the growth period. I am inclined to believe that it necessarily does so.

This act of synapsis is one that occurs only in the germ-cells, and to me it has always seemed of the utmost importance. Let us consider the conditions of the process a little more fully, and see if something of value may not be gained in our search for the cause of differentiation. The primordial germ-cells have been more or less intimately a part of the body. Their double sets of chromosomes have been functioning individually, if not even antagonistically, and have built up cell-bodies of considerable size. These early germ-cells are then gathered together in a single place and are thereupon removed from so intimate relations with the somatic cells. Under these conditions, as we have noticed, they reproduce rapidly with constant decrease in the amount of cytoplasm and end up with cells almost entirely nuclear in proportion and strongly chromatic. Here division ceases and the opposing paternal and maternal chromosomes, their cytoplasmic environment practically gone, unite together in synapsis.

Conditions are evidently ripe for a change. The change becomes apparent in an altered behavior of the cell, which, no longer expending its energies in reproduction, grows enormously both in nucleus and cytosome. Reproduction has given place to constructive metabolism. This is, however, such a metabolism as finds expression nowhere else in the life-cycle of organisms. Morphologically, at least, it accompanies a condition in which the paternal and maternal chromosomes are reduced to common units. Physiologically, I believe, it is a state wherein the chromosomes, having passed through many generations of cells in a cytoplasmic environment peculiar to the particular organism of which they are members, and having possibly exhausted the metabolic resources of these conditions, unite their common energies and construct a new cytoplasm. The extent of this growth varies with the species, but in every case is con-

siderable, and is accompanied, or rather preceded, by a corresponding nuclear enlargement. This growth in the case of the ovum is much more extensive, including the formation of all the varieties of differentiated "stuffs," but takes place under similar conditions. In both sexes this unique state of the cell is terminated by two mitoses, one of which witnesses the separation of the paired homologous chromosomes and their distribution into different cells. This, in reality, is the beginning of a new individual, for here are new conditions throughout the cell. It seems possible to me, in the light of our present knowledge of animal development, to gain some idea of the general nature of the phenomena involved in maturation and fertilization, and to grasp something of the meaning of the cell changes that take place at these periods. Clearly enough, in maturation there is a separation of chromosomes and in fertilization a restoration to the normal number. These alternately joined and separated chromosome groups are of different sexual origin and distribution, but are themselves without sexual characters, for in one generation they may be in a male organism and in the next in a female.

But when we have recorded these important facts we have by no means exhausted our knowledge of the difference between the immature and mature germ-cell. Much important work has been done within recent years upon the organization of the egg, and it has been clearly demonstrated that this is of a high order of complexity. The recognition of different "stuffs" in the egg, and the discovery that they are of various organ-forming powers, show most clearly that our theories of development must take the cytoplasm into due consideration. Such a recognition awarded cytoplasmic localization lessens in no measure the importance of the chromosomes, a fact that has been appreciated by none more clearly than by those who are most familiar with egg organization. Experimentally it has been proved that these particular materials are able to develop the earlier stages of the embryo without cell formation or nuclear division; and yet those who have most knowledge of such processes do not minimize the importance of the chromosomes, even though it may be granted that the cytoplasm has much to do with conditioning the early stages of development. Nor does the additional fact that the final stages of somatic differentiation are characterized by the preeminence of the cytoplasm weaken our belief in the primary importance of the chro-

mosomes. I think that a reconciliation of the apparently contradictory facts of cytoplasmic localization and chromosome control is not at all impossible.

There is but one time in the history of an organism when the production and arrangement of these specific materials occur; there is but one time when the biparental chromosomes function in common and not as separate entities. These periods are coincident, and I cannot escape the conviction that they are related as effect to cause. We find the spermatogonia and oogonia reduced by repeated divisions to an almost acytoplasmic condition, and at that point discover the chromosomes pairing off. During a long period of association, in certain grasshoppers extending through the winter months, these chromosomes remain together, and, at the end, present themselves to us as members of an entirely different cell, with a large, clear nucleus and ample cytosome. In the egg the contrast between stages is even more remarkable, but here also the nucleus grows with the cytoplasm.

From these observations we must conclude that if the nucleus governs metabolism, and many observations tell us this is true, the unusual condition of the nucleus is the cause for the unusual growth of the cytosome. The possibilities for the new growth are established by the unified activities of the chromosomes from the two parents, I would conclude. But the cytoplasmic growth is only one aspect of the change that has been taking place at this time. The chromosomes themselves have been active participants in a series of interactions that leave them modified in structure and function in many cases, the only exception being such as govern Mendelian characters. Undoubtedly the nature and amount of this interaction vary between the same chromosomes in different generations, and herein is the cause of variation. Once the chromosomes are separated in maturation, however, their character is determined and they do not again alter in the germ-cycle until another synapsis. I believe these views are established by common observation. The descendants of two parents rarely develop body characters much alike, but now and then real twins appear and they are strikingly alike. The reasons for this are not far to seek, for the twins are the product of the same two germ-cells, while ordinary children are from different cells in each parent.

From the plant kingdom we may gain still further evidence

of the same nature. Here, where vegetative reproduction may be carried on for long periods of time, there are forms that suffer practically no variation at all under these conditions; but allow them to propagate sexually and variation occurs as usual. In a self-fertilized plant the material comes from one source in both cases, but in vegetative propagation the same double set of chromosomes reproduces itself constantly unchanged; but in sexual reproduction there is the interaction of chromosomes in synapsis and the formation of a new cytoplasm that is lacking otherwise. The fundamental importance of the germ-cell organization is thus indisputably proved.

With the chromosomes in the role of character determinants, how then may we regard the operation of the cell parts? We must, in the first place, I think, consider the cyclical character of cell division. Nucleus and cytosome are physically and chemically unlike structures, separated by a thin membrane. There can be no doubt that the ordinary phenomena of osmosis find a place with consequent interchange of materials. After a cell division the cytosome grows, the nucleus grows, and the chromatin doubles its volume. New material has been added and transformed into the likeness of the old. Experiments teach us that the presence of the nucleus is required for the operation of these changes. Then comes another mitosis. The nucleus as a discrete body disappears and its protoplasm merges with that of the cytosome. Thereupon there is formed the familiar bipolar figure and the chromosomes are accurately divided along a plane established before the breaking down of the nucleus. This occurs in the spermatogonia, where the cytoplasm is reduced in amount to such a degree that so minute a spindle is produced that it can scarcely be distinguished among the chromosomes. The division of the chromosomes is the final effort of these cells. That such a process would produce equivalent daughter-cells seems obvious. A similar occurrence obtains in the reproduction of somatic cells, but there is an important difference between the two categories. In the division of the spermatogonia there is a constant increase of the chromatin at the expense of the cytoplasm, resulting in cells a very large proportion of which individually is chromatin, while in somatic mitoses the cytoplasm enlarges disproportionately to the nucleus and its chromatin. This cytomorphosis seems to exhaust the possibilities of differential interchange between nucleus and cytosome, and fixes the character of the cell.

In the fertilized egg the conditions are much different. Preceding the entrance of the spermatozoon the synaptic chromosomes have operated in the process of building up both a large nucleus and a large cytosome. At maturation most of the nonchromatic material is discharged into the cytosome, so that upon their conjugation the pronuclei are probably equivalent in other respects aside from the chromosomes. It has been shown that in the early stages of embryo formation there is an actual contribution of chromosomic material to the cytoplasm. That the chromosomes increase in size after each division by taking up material from the cytoplasm is a common observation. There is thus a constant circulation of material through nucleus and cytosome, and that, I consider, offers an adequate explanation of the *means* of differentiation, for if the chromosomes were individually different they would respond adaptively under the varying conditions of development.

Assuming this as an explanation of the means of differentiation, how can we conceive the operation of the processes of development in relation to the germ-cells, which divide at the same time as the body-cells and yet suffer no differentiation? The only observations that would serve as a key to this problem would indicate that the absence of differentiation is due to the retention of the entire chromosome complex unchanged. If differentiation is due to the interchange of material between the chromosomes and cytoplasm under like conditions, it would naturally follow that the absence of differentiation might result were there no such interchange. The few observations that we have would support this view. We are, however, much in need of a thorough knowledge of the changes undergone by all the cell elements during ontogenetic development. We would have a much broader foundation for our theories if we knew the nature of the chromosome complex in the various cells of the body as these became differentiated. It would then be possible to say, with more assurance than our present scanty observations incline us to do, that the germ-cells preserve the chromosome complex unaltered, while it becomes progressively and variously changed during histogenesis.

It is very clear that we cannot look to any variation in the operation of mitosis as a cause for somatic differentiation, since our observations indicate that the process is designed to produce exact chromosome equivalents. If the chromosomes are the controlling centers of differentiation, then the only con-

ception of their action that we can entertain is that they bear definite and progressively different relations to their cytoplasmic environment. Differentiation, we should therefore be inclined to say, is due to a series of adjustments between two constantly varying and interdependent structures, nucleus and cytosome, with the chromosomes acting as the measure of their interaction.

There has been in reality but one thing postulated as a basis for my views regarding the organization of the chromosomes, viz., that they are specific, self-perpetuating morphological units. If this be granted, synapsis, segregation and double chromosome groups necessarily are true conceptions, for there is no other way of accounting for the reduction in the number of chromosomes in mauration and the restoration of this number in fertilization. Once it is conceded that the chromosomes are self-perpetuating cell units, then there can be no questioning the fact of a reducing or segregating division, for if a set of chromosomes from the father enters the egg and duplicates a set already present no other explanation of the reappearance of the same two sets in the mature germ-cells can be offered. The whole question of heredity based upon chromosome structure centers here. Every fact that makes for the establishment of the chromosomes as definite structural elements is an argument in favor of a reducing or segregating division. *The loss of the identity of the chromosomes in any period of cell proliferation means the recreation of the chromosomes in the next mitosis.* This fact must be clearly realized. If they are not continuous structures they are new structures in each cell. If they are *new* structures, then it must be explained how they reappear in the same number, size and form from generation to generation of cells and throughout the species, genus and family of the grasshoppers. To say that they form anew after each mitosis is to postulate an organization outside of themselves that controls their reintegration. This merely begs the question, for we know of no such organization. I maintain that the discovery of the same group of chromosomes in all the species of *Hesperotettix* is alone sufficient to establish the fact that they are self-perpetuating individuals. We actually see them reproducing themselves in one mitosis after another, and we find them invariably in every animal that we study.

Now, in arguing thus for the continuity of the chromosomes, it is not intended to convey the idea that they are always of

exactly the same size, form and physical constitution, nor that there is not addition to, or change in, the material substance of which they are composed. They are no more immutable or invariable than the grasshopper which at one time is an egg, at another a nymph, and finally a full-winged adult. I would as soon question the individuality of one as of the other, on the argument that they cannot always be distinguished in the same form. Enough evidence has now been developed by various cytological and experimental researches to show that there is a fixity and definiteness in chromosome organization sufficient to support adequately what is commonly known as the theory of the "individuality of the chromosomes." The burden of proof rests strongly upon those who oppose this theory, and to remove it they will have to offer some definite explanation for the appearance of such a structure as the hexad multiple of *Hesperotettix*.

The mere fact that the chromatin granules diffuse through the nucleus so that the outlines of the individual chromosomes cannot be distinguished is no argument against the persistence of the chromosomes. Neither can I regard the occurrence of occasional cases of amitosis as in the least weakening the theory of chromosome individuality. It may teach us more about chromosomes to learn that under some circumstances they may show such a suspension of visible integration as amitosis would indicate, but we shall first have to know a great deal more about amitosis than we do now, and we shall want to know how much the parasitical nature of the forms in which direct division usually occurs has had to do with the modification of all other organic phenomena. In other words, I maintain that other than negative evidence will have to be offered in explanation of the occurrence of the same number of chromosomes throughout the family *Acrididæ*, and of the same combination that characterizes certain genera. If these are not self-perpetuating structures then there is some agent outside which determines their being, and it will be necessary to demonstrate this before the simpler self-integration theory is abandoned.

I am quite prepared to admit also that in one species even there may be a variation in the integration of the chromatin material, resulting in some numerical variation of the chromosomes, without losing my belief in the necessity for this defi-

niteness in the grasshoppers. We do not yet know how much difference there may be in the organization of the various chromosomes of a complex nor how variable in importance they may be. We know that eggs differ much in the extent of their organization, so that their potencies are entirely different; and since the chromosomes represent a part of this organization they also may vary in some forms. There may also be more than the duplicate set of chromosomes present, which would presumably do no more than bring about greater variation or minor differences in the distribution of characters.

Taxonomy has sought to determine the true relationship between animals and to divine the lines of their descent. As criteria they have used, in the main, structural peculiarities, and these have been considered of different values, depending upon the number and variety of forms to which they are common. We speak in this way of family, generic and specific characters. For instance, in the Orthoptera we distinguish the family *Acrididæ*, which has as recognition characters strongly developed jumping legs, vertical head, short antennæ, three ocelli, three-jointed tarsi, auditory organs on the basal segment of the abdomen, etc. An examination of these characters will show that it is not the presence of any peculiar structure that distinguishes this group of Orthoptera from other Orthoptera, but it is the nature and extent of structural development that is distinctive. All insects have, for instance, a third pair of legs, a pair of antennæ, a head, etc., but in the *Acrididæ* it is the particular proportion of the third pair of legs, the length of the antennæ, and the position of the head with reference to the thorax that marks off this group from others. It is the entire organization of the acridian body that differs from representatives of other orthopteran families, and these features of external structure that taxonomists have selected are merely very striking ones used for diagnostic purposes.

Not a cell in the body of an acridian is like that of a locustid, nor are any of the organs similar. The structure of the testis, for instance, is so dissimilar that a glance at a section through a low power of the microscope is sufficient to distinguish the source of the preparation without counting the chromosomes. I have therefore come to the conclusion that for accurate, systematic work, not only will the external anatomy have to be studied but also the internal. Ordinarily, the correlation of

parts is sufficiently close to allow the use of purely external structures, but in other instances the entire anatomy will have to be understood. It has been a matter of great interest to me to find how real are the distinctions between species that have been established by systematists. In the genus *Hesperotettix*, that I have been using as an example, the species are clearly marked by peculiarities of external structure, color, etc., and these are strictly correlated with germ-cell characters.

These things all speak strongly to me of definiteness and thoroughness of organization, a conception that we ordinarily express as "correlation of characters." It means, in truth, that an organism is a very complex assemblage of parts, each of which at any particular time has a definite form and relation to the other parts. To know it thoroughly we must be familiar with *all* its parts at that particular stage which it has reached in its development. An organism is an organism, no matter at what ontogenetic period it is regarded. Practically all crustaceans, as we know, at one stage of their existence have a form called a "nauplius." The definitive organization may express itself by only slight modifications of the early type of structure, or it may progressively alter its form until it bears no resemblance to the former condition. But these nauplii are not all alike, and doubtless every form has a different expression for this structural type could we but distinguish it. Not only is this true, but in all probability every cell is characteristically different.

With this conception of organisms we see that in their study the distinction between cytology and taxonomy is not great. Were our knowledge of cell structure in the grasshopper complete enough we might erect a system of classification based upon cytological characters, just as reasonably as we have designated one using external anatomical structures. All of which goes to show that organisms at all stages of development and in all their parts are specifically constituted. It is the peculiar privilege of cytology not only to recognize these differences but to determine the means by which they came about. The apprehension of large principles of organization should therefore be our aim, and I have no doubt that once an understanding of the cytological changes in the body of an animal during its ontogeny is reached we shall have solved, as far as it is possible for us to do, some of the larger problems of heredity and development that have become our scientific inheritance.